

CLAIMS

Claims as Pending:

118. (previously amended): A method of inhibiting expression of an endogenous cellular gene in a cell, the method comprising the step of:

contacting a first target site in the endogenous cellular gene with a first zinc finger protein, wherein said first zinc finger protein is engineered and wherein the Kd of the zinc finger protein is less than about 25 nM;

thereby inhibiting expression of the endogenous cellular gene.

119. (previously added): The method of claim 118, wherein the step of contacting further comprises contacting a second target site in the endogenous cellular gene with a second zinc finger protein.

120. (previously added): The method of claim 119, wherein the first and second target sites are adjacent.

121. (previously added): The method of claim 120, wherein the first and second zinc finger are covalently linked to form a fusion protein.

122. (previously added): The method of claim 118, wherein the first zinc finger protein is a fusion protein comprising a regulatory domain.

123. (previously added): The method of claim 122, wherein the first zinc finger protein is a fusion protein comprising at least two regulatory domains.

124. (previously added): The method of claim 119, wherein the first and second zinc finger proteins are fusion proteins, each comprising a regulatory domain.

125. (previously added): The method of claim 124, wherein the first and second zinc finger protein are fusion proteins, each comprising at least two regulatory domains.

126. (previously added): A method of inhibiting expression of an endogenous cellular gene in a cell, the method comprising the step of:

contacting a target site in the endogenous cellular gene with an engineered fusion zinc finger protein comprising six fingers and a regulatory domain, wherein the Kd of the zinc finger protein is less than about 25 nM;

thereby inhibiting expression of the endogenous cellular gene.

127. (previously added): The method of claim 118, wherein the cell is selected from the group consisting of an animal cell, a plant cell, a bacterial cell, a protozoal cell, and a fungal cell.

128. (previously added): The method of claim 127, wherein the cell is a mammalian cell.

129. (previously added): The method of claim 128, wherein the cell is a human cell.

130. (previously added): The method of claim 118, wherein expression of the endogenous cellular gene is inhibited by at least about 20%.

131. (previously added): The method of claim 118, wherein the endogenous cellular gene is selected from the group consisting of VEGF, ER*, IGF-1, c-myc, c-myb, ICAM, and Her2/Neu.

132. (previously added): The method of claim 131, wherein the endogenous cellular gene is VEGF.

133. (previously added): The method of claim 118, wherein the inhibition of gene expression prevents gene activation.

134. (previously added): The method of claim 122, wherein the regulatory domain is selected from the group consisting of a transcriptional repressor, an endonuclease, a methyl transferase, and a histone deacetylase.

135. (previously added): The method of claim 124, wherein the regulatory domain is selected from the group consisting of a transcriptional repressor, an endonuclease, a methyl transferase, and a histone deacetylase.

136. (previously added): The method of claim 118, wherein the method further comprises the step of first administering to the cell a delivery vehicle comprising the zinc finger protein, wherein the delivery vehicle comprises a liposome or a membrane translocation polypeptide.

137. (previously added): The method of claim 118, wherein the target site is upstream of a transcription initiation site of the endogenous cellular gene.

138. (previously added): The method of claim 118, wherein the target site is adjacent to a transcription initiation site of the endogenous cellular gene.

139. (previously added): The method of claim 118, wherein the zinc finger protein comprises an SP-1 backbone.

140. (previously added): The method of claim 139, wherein the zinc finger protein comprises a regulatory domain and is humanized.

141. (previously amended): A method of activating expression of an endogenous cellular gene, the method comprising the step of:

contacting a first target site in the endogenous cellular gene with a first zinc finger protein, wherein said first zinc finger protein is engineered and wherein the Kd of the zinc finger protein is less than about 25 nM;

thereby activating expression of the endogenous cellular gene.

142. (previously added): The method of claim 141, wherein the step of contacting further comprises contacting a second target site in the endogenous cellular gene with a second zinc finger protein.

143. (previously added): The method of claim 142, wherein the first and second target sites adjacent.

144. (previously added): The method of claim 143, wherein the first and second zinc finger proteins are covalently linked to form a fusion protein.

145. (previously added): The method of claim 141, wherein the first zinc finger protein is a fusion protein comprising a regulatory domain.

146. (previously added): The method of claim 145, wherein the first zinc finger protein is a fusion protein comprising at least two regulatory domains.

147. (previously added): The method of claim 142, wherein the first and second zinc finger proteins are fusion proteins, each comprising a regulatory domain.

148. (previously added): The method of claim 147, wherein the first and the second zinc finger protein are fusion proteins, each comprising at least two regulatory domains.

149. (previously added): A method of activating expression of an endogenous cellular gene, the method comprising the step of:

contacting a target site in the endogenous cellular gene with an engineered fusion zinc finger protein comprising six fingers and a regulatory domain, wherein the K_d of the zinc finger protein is less than about 25 nM;

thereby activating expression of the endogenous cellular gene.

150. (previously added): The method of claim 141, wherein the cell is selected from the group consisting of an animal cell, a plant cell, a bacterial cell, a protozoal cell, and a fungal cell.

151. (previously added): The method of claim 150, wherein the cell is a mammalian cell.

152. (previously added): The method of claim 151, wherein the cell is a human cell.

153. (previously added): The method of claim 141, wherein expression of the endogenous cellular gene is activated to at least about 150%.

154. (previously added): The method of claim 141, wherein the endogenous cellular gene is selected from the group consisting of FAD2-1, EPO, GM-CSF, GDNF, VEGF, and LDL-R.

155. (previously added): The method of claim 154, wherein the endogenous cellular gene is VEGF.

156. (previously added): The method of claim 141, wherein the activation of gene expression prevents repression of gene expression.

157. (previously added): The method of claim 145, wherein the regulatory domain is selected from the group consisting of a transcriptional activator and a histone acetyltransferase.

158. (previously added): The method of claim 147, wherein the regulatory domain is selected from the group consisting of a transcriptional activator and a histone acetyltransferase.

159. (previously added): The method of claim 141, wherein the target site is upstream of a transcription initiation site of the endogenous cellular gene.

160. (previously added): The method of claim 141, wherein the target site is adjacent to a transcription initiation site of the endogenous cellular gene.

161. (previously added): The method of claim 141, wherein the zinc finger protein comprises an SP-1 backbone.

162. (previously added): The method of claim 161, wherein the zinc finger protein comprises a regulatory domain and is humanized.

163. (previously amended): A method of modulating expression of an endogenous cellular gene in a cell, the method comprising the step of:

contacting a first target site in the endogenous cellular gene with a first zinc finger protein, wherein said first zinc finger protein is engineered;
thereby modulating expression of the endogenous cellular gene.

164. (previously added): The method of claim 163, wherein the step of contacting further comprises contacting a second target site in the endogenous cellular gene with a second zinc finger protein.

165. (previously added): The method of claim 164, wherein the first and second target sites are adjacent.

166. (previously added): The method of claim 165, wherein the first and second zinc finger proteins are covalently linked to form a fusion protein.

167. (previously added): The method of claim 163, wherein the first zinc finger protein is a fusion protein comprising a regulatory domain.

168. (previously added): The method of claim 167, wherein the first zinc finger protein is a fusion protein comprising at least two regulatory domains.

169. (previously added): The method of claim 164, wherein the first and second zinc finger proteins are fusion proteins, each comprising a regulatory domain.

170. (previously added): The method of claim 169, wherein the first and second zinc finger protein are fusion proteins, each comprising at least two regulatory domains.

171. (previously added): A method of modulating expression of an endogenous cellular gene in a cell, the method comprising the step of:

contacting a target site in the endogenous cellular gene with an engineered fusion zinc finger protein comprising six fingers and a regulatory domain;
thereby modulating expression of the endogenous cellular gene.

172. (previously added): The method of claim 163, wherein the cell is selected from the group consisting of an animal cell, a plant cell, a bacterial cell, a protozoal cell, and a fungal cell.

173. (previously added): The method of claim 172, wherein the cell is a mammalian cell.

174. (previously added): The method of claim 173, wherein the cell is a human cell.

175. (previously added): The method of claim 163, wherein the endogenous cellular gene is selected from the group consisting of VEGF, ER*, IGF-1, e-myc, c-myb, ICAM, Her2/Neu, FAD2-1, EPO, GM-CSF, GDNF, and LDL-R.

176. (previously added): The method of claim 175, wherein the endogenous cellular gene is VEGF.

177. (previously added): The method of claim 167, wherein the regulatory domain is selected from the group consisting of a transcriptional repressor, a transcriptional activator, an endonuclease, a methyl transferase, a histone acetyltransferase, and a histone deacetylase.

178. (previously added): The method of claim 169, wherein the regulatory domain is selected from the group consisting of a transcriptional repressor, a transcriptional activator, an endonuclease, a methyl transferase, a histone acetyltransferase, and a histone deacetylase.

179. (previously added): The method of claim 163, wherein the method further comprises the step of first administering to the cell a delivery vehicle comprising the zinc finger protein, wherein the delivery vehicle comprises a liposome or a membrane translocation polypeptide.

180. (previously added): The method of claim 163, wherein the target site is upstream of a transcription initiation site of the endogenous cellular gene.

181. (previously added): The method of claim 163, wherein the target site is adjacent to a transcription initiation site of the endogenous cellular gene.

182. (previously added): The method of claim 163, wherein the zinc finger protein comprises an SP-1 backbone.

183. (previously added): The method of claim 182, wherein the zinc finger protein comprises a regulatory domain and is humanized.